

Claims

What is claimed is:

1. A method for designing a multiplicity of primers for simultaneous
5 amplification of a multiplicity of target DNA fragments in a single multiplex polymerase
chain reaction comprising the steps of:
 - a. aligning a first primer and a second primer; and
 - b. selecting the first primer wherein 1) the first primer at its 3' end does not contain
four or more bases that are perfectly matching to the 3' end sequence of the first primer or a
10 second primer; the first primer at its 3' end does not contain seven or more bases that are
perfectly matching except one mismatch to the 3' end sequence of the first primer or the
second primer; the first primer at its 3' end does not contain six or more bases that are
perfectly matching to a sequence anywhere of the first primer or the second primer; and the
first primer at its 3' end does not contain eleven or more bases that are perfectly matching
15 except one mismatch to a sequence anywhere of the first primer or the second primer.
2. A method of claim 1 wherein at least 100 primers are designed.
3. A method of claim 2 wherein at least 200 primers are designed.
4. A method of claim 3 wherein at least 1000 primers are designed.
5. A method of claim 1 wherein at least 50 target DNA fragments are produced
20 in the single multiplex polymerase chain reaction.
6. A method of claim 1 wherein at least 100 target DNA fragments are produced
in the single multiplex polymerase chain reaction.

7. A method of claim 1 wherein at least 500 target DNA fragments are produced in the single multiplex polymerase chain reaction.

8. A method of claim 1 wherein the single multiplex polymerase chain reaction is used for an application.

5 9. A method of claim 8 wherein the application is selected from the group consisting of an identification of multiple genes related to multifactorial diseases, a genome-scale detection of genetic alterations in cancers, a study in large-scale pharmacogenetic reactions, a genotyping genetic polymorphism in a large population, and a gene expression profiling.

10 10. A method of claim 1 wherein the primers increase the efficacy of the single multiplex polymerase chain reaction.

11. A method of claim 1 wherein the primers minimize the non-specific extension of the single multiplex polymerase chain reaction.

12. A computer product comprising a computer readable medium containing a
15 computer program which once executed by a computer processor performs the method of claims 1-11.